

WHAT IS CLAIMED IS:

1. A human embryoid body-derived (EBD) cell culture that comprises cells at least some of which simultaneously express polypeptide or mRNA markers that are characteristic of at least two different cell types, wherein the cell types are selected from the group consisting of an ectodermal cell, a mesodermal cell, and an endodermal cell.
2. The culture of claim 1 wherein said cells simultaneously express characteristic polypeptide or mRNA markers characteristic of an ectodermal cell, a mesodermal cell, and an endodermal cell.
3. The culture of claim 1 wherein said cells simultaneously express an ectodermal cell marker selected from the group consisting of nestin, vimentin, neurofilament light isoform, microtubule-associated protein 2c, tau, nonphosphorylated neurofilament heavy isoform, neuron-specific enolase, tyrosine hydroxylase, glial fibrillary acidic protein, CNPase, and galactocerebroside.
4. The culture of claim 3 wherein said cells simultaneously express an ectodermal cell marker selected from the group consisting of nestin, vimentin, and glial fibrillary acidic protein.
5. The culture of claim 3 wherein said cells simultaneously express an ectodermal cell marker selected from the group consisting of nestin and vimentin.
6. The culture of claim 1 wherein said cells simultaneously express a mesodermal cell marker selected from the group consisting of myf6, myosin lightchain 2 ventricular isoform, and flk1 .
7. The culture of claim 1 wherein said cells simultaneously express an endodermal cell marker selected from the group consisting of α -1-fetoprotein and GATA-4.

09757431.100101

8. The culture of claim 1 wherein said cells simultaneously express a first marker selected from the group consisting of nestin and vimentin and a second marker selected from the group consisting of myf6, myosin light-chain 2 ventricular isoform, flk1, α -1-fetoprotein and GATA-4.
9. The culture of claim 1 that under suitable cell culture conditions proliferates for at least thirty population doublings without being immortal under said conditions.
10. The culture of claim 9 that under suitable cell culture conditions proliferates for at least sixty population doublings.
11. The culture of claim 1 that proliferates under cell culture conditions that are nonpermissive for proliferation of human embryonic germ cells.
12. The culture of claim 11 that proliferates in a media that lacks leukemia inhibitory factor, a fibroblast feeder layer, or both.
13. The culture of claim 1 wherein said cells are transfectable with a retrovirus or a lentivirus or both.
14. The culture of claim 1 that does not cause formation of a teratoma when injected into a SCID mouse.
15. The culture of claim 1 that is clonal.
16. The culture of claim 15 that is clonally derived from a single EBD cell.
17. A human EBD cell culture that comprises cells at least some of which simultaneously express a first polypeptide or mRNA marker selected from the group consisting of nestin and vimentin and a second polypeptide or mRNA marker selected from the group consisting of myf6, myosin light-chain 2 ventricular isoform, flk1, α -1-fetoprotein and GATA-4, wherein under suitable cell culture conditions, the culture proliferates for at least thirty population doublings.

09767421-100101

18. A human EBD cell culture that comprises cells at least some of which simultaneously express polypeptide or mRNA markers that are characteristic of at least two different cell types, wherein the cell types are selected from the group consisting of an ectodermal cell, a mesodermal cell, and an endodermal cell, wherein under suitable cell culture conditions the culture proliferate for at least thirty population doublings, and wherein the culture does not cause formation of a teratoma when injected into a SCID mouse.

19. A human EBD cell culture that comprises cells at least some of which (a) proliferate for at least thirty population doublings under suitable cell culture conditions; (b) proliferate in a media that lacks leukemia inhibitory factor, a fibroblast feeder layer, or both; (c) are transfectable with a retrovirus or lentivirus or both; and (d) do not cause formation of a teratoma when injected into a SCID mouse.

20. The culture of claim 19 wherein said cells simultaneously express polypeptide or mRNA markers that are characteristic of at least two different cell types, wherein the cell types are selected from the group consisting of an ectodermal cell, a mesodermal cell, and an endodermal cell.

21. The culture of claim 19 wherein said cells simultaneously express a first polypeptide or mRNA marker selected from the group consisting of nestin and vimentin and a second polypeptide or mRNA marker selected from the group consisting of myf6, myosin light-chain 2 ventricular isoform, flk1, α -1-fetoprotein and GATA-4.

22. A method of making a human EBD cell culture comprising:

(a) culturing human embryonic germ cells under conditions that are suitable for formation of cystic embryoid bodies,

(b) dissociating the cystic embryoid bodies to provide a constituent cell, and

(c) culturing the constituent cell under conditions suitable to produce a human EBD cell culture that comprises cells at least some of which simultaneously express polypeptide or mRNA markers that are characteristic of at least two different cell types, wherein the cell types are selected from the group consisting of an ectodermal cell, a mesodermal cell, and an endodermal cell.

09757431-100101

23. The method of claim 22 comprising selecting a single EBD cell from the EBD cell culture and culturing the single EBD cell to produce a clonal EBD cell culture.
24. The method of claim 22 comprising culturing the constituent cell in a media comprising human basic fibroblast growth factor.
25. The method of claim 24 comprising culturing the constituent cell in a media selected from the group consisting of RPMI 1640 supplemented with 15% FCS and EGM2MV.
26. The method of claim 25 comprising culturing the constituent cell in EGM2MV media.
27. The method of claim 22 comprising culturing the constituent cell on a matrix.
28. The method of claim 27 comprising culturing the constituent cell on a matrix that is selected from the group consisting of collagen I, human extracellular matrix, and tissue culture-treated plastic.
29. The method of claim 28 comprising culturing the constituent cell on a matrix selected from the group consisting of collagen I and human extracellular matrix.
30. The method of claim 22 comprising culturing the constituent cell on a media that is not permissive for proliferation of the EG cells.
31. The method of claim 30 comprising culturing the constituent cell on a media lacking leukemia inhibitory factor, a fibroblast feeder layer, or both.
32. The method of claim 22 comprising culturing the EBD cell culture for at least 30 population doublings.

096749-1001

33. A method of treating a human disease or injury comprising introducing a composition comprising an EBD cell or EBD cell culture into the body of a patient having **the disease or injury**.

09/6/21 10:10:10